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Protein Synthesis During Fungal Spore Germination: Differential Protein Synthesis During Germination of *Botryodiplodia theobromae* Spores[1]

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The preformed messenger ribonucleic acid in *Botryodiplodia theobromae* spores directs the synthesis of several relatively stable polypeptides.

Reports from several laboratories have concluded that fungal spores contain preformed messenger ribonucleic acid (mRNA) (1-6, 8, 10). During the initial phases of germination of *Botryodiplodia theobromae* conidiospores, translation is temporally separated from transcription since protein synthesis occurs in the absence of detectable RNA synthesis (2). This paper reports experiments to determine whether the spore mRNA directs the synthesis of more than one soluble protein and whether the proteins synthesized early in the germination process differ from those synthesized at a later stage.

B. theobromae spores were grown, harvested, and germinated at 1 mg of spores/1 ml of medium as described previously (2). Protein synthesis was determined by incubating the spores with a mixture of 12 ^{3}H-amino acids or with an identical mixture of ^{14}C-amino acids. Each ^{3}H-amino acid had a specific activity of 1 Ci/mmole; each ^{14}C-amino acid had a specific activity of 100 mCi/mmole. The cells were disrupted as described previously (2) in a buffer composed of 0.2 M tris(hydroxymethyl)-aminomethane (*p*H 7.8), 0.4 M sucrose, 0.05 M NH_4Cl, 0.005 M $MgCl_2$, and 0.005 M 2-mercaptoethanol. The extract was centrifuged at 20,000 × *g* for 20 min and the supernatant at 30,000 × *g* for 30 min. The ensuing supernatant was treated with protamine sulfate (2 mg/ml of extract) for 30 min to precipitate ribosomes and RNA. After centrifugation, protein in the soluble fraction was

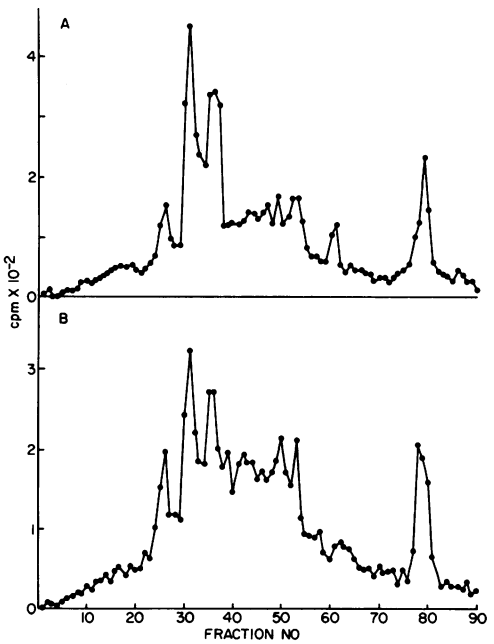

FIG. 1. *Distribution of radioactivity in the soluble polypeptides synthesized during the first hour of germination of B. theobromae spores. One liter of the spore suspension was pulsed from 0 to 1 hr with 100 μCi of a mixture of 12 ^{3}H-amino acids. The spore suspension was harvested and divided into two equal samples. One sample was immediately frozen (A), and the other was returned to fresh medium containing 12 unlabeled amino acids (each at 0.002 M) and incubated an additional 4 hr before harvesting (B). The soluble protein fraction was isolated and electrophoresed as described in the text.*

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precipitated with trichloroacetic acid (final concentration of 5% [w/v]) for at least 1 hr at 0 C. The precipitate (soluble protein fraction), which contained 5 to 8% of the radioactivity taken up by the spores, was dissolved in 0.5 ml of 4% (w/v) sodium dodecyl sulfate (SDS), 0.001 M 2-mercaptoethanol, and 0.1 M sodium phosphate, *p*H 7.0, by heating at 100 C for 10 min. Polyacrylamide gel electrophoresis was conducted on 10-cm 6% acrylamide gels in SDS as described by Maizel (7). The gels were quickly frozen with solid CO_2 and cut into 1-mm slices with a gel slicer. The radioactive material was eluted from the gel slices (11) and counted in a liquid scintillation spectrometer.

From the results depicted in Fig. 1A it is obvious that several soluble polypeptides are synthesized during the first hour of germination. Since RNA synthesis was undetected during the first hour of germination (2), the synthesis of these polypeptides is presumably directed by spore mRNA. Although the data are not shown, soluble protein obtained from spores labeled from 0 to 30 min produced a radioactive profile nearly identical to that reported in Fig. 1A. The proteins synthesized during the first hour of germination were quite stable since the radioactive profile of spores pulse labeled for 1 hr (Fig. 1A) was not significantly different from those pulsed for 1 hr followed by a chase period of 4 hr (90% germination) (Fig. 1B).

To determine if the soluble polypeptides synthesized early in the germination process were similar to or different from those synthesized during germ tube formation (a period of rapid RNA synthesis [2]) the spores were la-

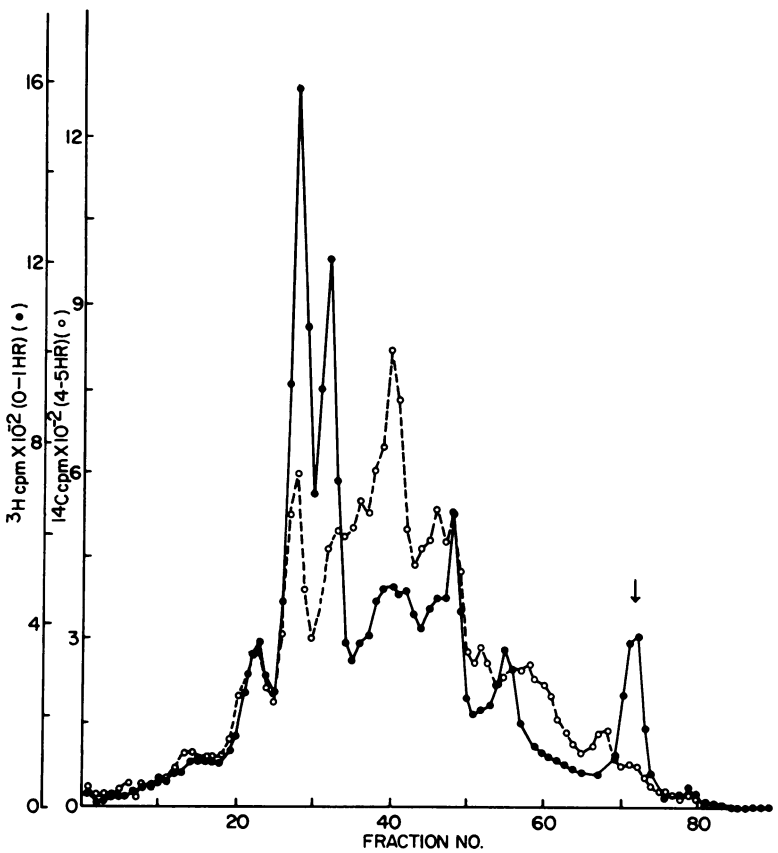

FIG. 2. *Distribution of radioactivity in the soluble polypeptides synthesized during two stages of germination of B. theobromae spores. A 500-ml sample of the spore suspension was incubated from 0 to 1 hr with 50 μCi of a mixture of 12 ^{3}H-amino acids. A separate 25-ml sample was incubated from 4 to 5 hr with 10 μCi of an identical mixture of 12 ^{14}C-amino acids. The ^{3}H-labeled and ^{14}C-labeled spores were combined prior to homogenization. A 500-μg amount of soluble protein, which contained 22,000 counts/min of ^{3}H-labeled protein (0 to 1 hr; ●) and 19,000 counts/min of ^{14}C-labeled protein (4 to 5 hr; ○), were subjected to electrophoresis.*

beled from 0 to 1 hr with the ^{3}H-amino acid mixture, and a separate sample was labeled from 4 to 5 hr with the ^{14}C-amino acid mixture. Because the rate of protein synthesis was more rapid from 4 to 5 hr than from 0 to 1 hr (2), a smaller quantity of spores were pulse labeled at the later stage of germination (see legend to Fig. 2). The amounts of spore material chosen for this experiment were such that the final extract contained approximately the same number of ^{14}C counts as ^{3}H counts. The results of a typical experiment are demonstrated in Fig. 2. The relative distribution of radioactivity in the soluble polypeptides synthesized during the two spore stages were quite different; the greatest difference was reflected in the synthesis of a rapidly migrating protein (depicted by the arrow in Fig. 2) which was synthesized to a greater extent from 0 to 1 hr. Using the procedure of Shapiro et al. (9), the molecular weight of this polypeptide was estimated to be between 11,000 and 13,000.

Three conclusions are apparent from the experiments presented: (i) the preformed mRNA in the spore contains the information necessary for the synthesis of several soluble polypeptides, (ii) most of the polypeptides synthesized early in germination are stable, and (iii) there is a difference in the relative distribution of the soluble polypeptides synthesized at two stages of germination.

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